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3 **Standard genotyping overestimates transmission of *Mycobacterium***
4 ***tuberculosis* among immigrants in a low incidence country**

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56

57 **ABSTRACT**

58

59 Immigrants from high tuberculosis (TB) incidence regions are a risk group for
60 TB in low-incidence countries such as Switzerland. In a previous analysis of a
61 nationwide collection of 520 *Mycobacterium tuberculosis* isolates from 2000-2008, we
62 identified 35 clusters comprising 90 patients based on standard genotyping (24-loci
63 MIRU-VNTR and spoligotyping). Here, we used whole genome sequencing (WGS) to
64 revisit these transmission clusters. Genome-based transmission clusters were
65 defined as isolate pairs separated by ≤ 12 single nucleotide polymorphisms (SNPs).
66 WGS confirmed 17/35 (49%) MIRU-VNTR clusters; the other 18 clusters contained
67 pairs separated by >12 SNPs. Most transmission clusters (3/4) of Swiss-born
68 patients were confirmed by WGS, as opposed to 25% (4/16) of clusters involving only
69 foreign-born patients. The overall clustering proportion using standard genotyping
70 was 17% (90 patients, 95% confidence interval [CI]: 14-21%), but only 8% (43
71 patients, 95% CI: 6-11%) using WGS. The clustering proportion was 17% (67/401,
72 95% CI: 13-21%) using standard genotyping and 7% (26/401, 95% CI: 4-9%) using
73 WGS among foreign-born patients, and 19% (23/119, 95% CI: 13-28%) and 14%
74 (17/119, 95% CI: 9-22%), respectively, among Swiss-born patients. Using weighted
75 logistic regression, we found weak evidence for an association between birth origin
76 and transmission (aOR 2.2, 95% CI: 0.9-5.5, comparing Swiss-born patients to
77 others). In conclusion, standard genotyping overestimated recent TB transmission in
78 Switzerland when compared to WGS, particularly among immigrants from high TB
79 incidence regions, where genetically closely related strains often predominate. We
80 recommend the use of WGS to identify transmission clusters in low TB incidence
81 settings.

82

83 **INTRODUCTION**

84

85 Tuberculosis (TB) remains an important public health concern in European
86 countries (1–3). Immigrants from high TB-incidence countries and HIV-infected
87 populations are common risk groups in Switzerland, as in other European countries
88 (4–8). We and others have previously shown that transmission of *Mycobacterium*
89 *tuberculosis* occurs, but is not more common among immigrants than in the native
90 population (9–12).

91 Mycobacterial interspersed repetitive-unit–variable-number tandem-repeat
92 (MIRU-VNTR), combined with spoligotyping (13, 14), remains the most commonly
93 used genotyping method in molecular epidemiology of TB (15). However, MIRU-
94 VNTR may not distinguish between genetically closely related strains despite the
95 absence of close epidemiological links between patients (16–18). MIRU-VNTR may
96 be suboptimal to study transmission among immigrants from high TB incidence
97 countries, where genetically closely related strains circulate over extended periods of
98 time (19). Hence, recent transmission among immigrants is potentially overestimated,
99 but the extent of this phenomenon is largely unknown. In contrast to standard
100 genotyping methods, whole genome sequencing (WGS) provides an increased
101 resolution and has been used to study *M. tuberculosis* transmission (20–24). In this
102 study, we re-analyzed transmission clusters previously defined by MIRU-VNTR,
103 using WGS to assess transmission of *M. tuberculosis* among Swiss- and foreign-born
104 TB patients (9).

105

106

107 MATERIALS AND METHODS

108

109 Study setting and study population

110 In 2012, we conducted a nationwide study of the molecular epidemiology of TB
111 in Switzerland as a collaborative project between the Swiss HIV Cohort Study
112 (SHCS), the National Center for Mycobacteria, diagnostic microbiology laboratories,
113 departments of respiratory medicine and public health, and the Federal Office of
114 Public Health (www.tb-network.ch) (9, 25–28). The study setting was previously
115 described in detail (9). Briefly, all patients in the SHCS diagnosed with TB between
116 2000 and 2008 were enrolled (n=93). In addition, we included a random sample of
117 288 TB cases from the 4,221 culture-confirmed TB patients reported to the National
118 TB Surveillance Registry, and all drug-resistant TB cases reported in Switzerland
119 (n=167) during the same period (categories not mutually exclusive). 24-loci MIRU-
120 VNTR and spoligotyping were used for the molecular detection of transmission
121 clusters (9). In this follow-up study, we performed WGS on the 90 *M. tuberculosis*
122 isolates belonging to one of the 35 MIRU-VNTR/spoligotyping clusters.

123

124 Clinical data collection and definitions

125 The clinical data collection was previously described in detail (9). The clustering
126 proportion was determined by the “n” method expressed as the number of patients in
127 clusters divided by the total number of individuals (29). MIRU-VNTR clusters were
128 defined as a group of isolates with identical MIRU-VNTR and spoligotyping patterns
129 (9). In addition, we used IS6110-Restriction Fragment Length Polymorphism (RFLP)
130 patterns, when available from the National Center for Mycobacteria (30). RFLP has a
131 higher resolution than MIRU-VNTR, particularly in strains of the “Beijing” genotype.

132 Isolates with identical MIRU-VNTR and spoligotype patterns, but different IS6110
133 patterns, were considered as non-clustered. “Mixed” molecular clusters were defined
134 as clusters with Swiss-born and foreign-born individuals, or foreign-born individuals
135 from different continents.

136

137 **Whole genome sequencing and phylogenetic analyses**

138 We generated whole genome sequences for all 90 patient isolates identified as
139 part of MIRU-VNTR clusters (9). We used Illumina Nextera XT or TruSeq library
140 preparation kits and Illumina HiSeq, MiSeq or NextSeq devices (Illumina, San Diego,
141 CA) for WGS according to manufacturer’s instructions. Isolates were re-sequenced
142 when the mean read depth was below 20x. FastQ files from multiple sequencing runs
143 of the same isolate were merged. We used KvarQ for initial quality check,
144 determination of *M. tuberculosis* phylogenetic lineages and *in silico* spoligotyping
145 pattern, as previously described (31). We then mapped short sequencing reads to a
146 hypothetical *M. tuberculosis* ancestral genome (identical with H37Rv in structure, but
147 with maximum likelihood-inferred ancestral bases (32), with BWA 0.6.2 (33)).
148 Samtools 0.1.19 was used to call variants (SNPs). We only retained positions with a
149 read depth of $\geq 10\%$ and $\leq 200\%$ of the average read depth for the whole genome,
150 and a phred-scaled quality score of ≥ 30 . We excluded positions in known repetitive
151 regions (23), as well as SNPs in genes in which we have previously identified 50 bp
152 sequences with homologous sequences elsewhere on the genome (Supplementary
153 Table 1). We also excluded positions associated with drug resistance (31). For the
154 analyses of read/allele counts at particular genomic loci, we extracted the number of
155 high quality bases from Variant Call Format (VCF)-files with SNPeff/SNPst (34).

156

157 **Transmission networks based on SNP distances**

158 We generated an alignment of all variable positions across the 90 isolates. We
159 then calculated the raw genetic distances (number of SNPs) for each isolate pair in
160 each MIRU-VNTR cluster with the “Compute Pairwise Distances” function (using the
161 “Pairwise deletion” option) in MEGA 5.2.2 (35). We defined a MIRU-VNTR cluster as
162 a “true” transmission cluster if all isolate pairs in the cluster were separated by ≤ 12
163 SNPs. In a sensitivity analysis, we opted for a stricter definition, whereby a MIRU-
164 VNTR transmission cluster was considered as confirmed if at least one of its isolate
165 pairs was separated by ≤ 5 SNPs. These thresholds of 12 and 5 SNPs were
166 previously established by Walker *et al.* (17). We imported an alignment of the
167 variable genomic positions into popart (<http://popart.otago.ac.nz>) to generate median
168 joining networks. Networks were generated for all 35 transmission clusters identified
169 by standard genotyping (MIRU-VNTR and spoligotyping) (9).

170

171 **Statistical analysis**

172 We re-analyzed risk factors for transmission using the WGS-based (“true”)
173 cluster definition. We used weighted logistic regression models to obtain age- and
174 sex-adjusted odds ratios (aOR) for the probability of belonging to a true molecular
175 cluster. We used the Kruskal-Wallis rank sum test to assess differences between
176 mean genetic distances of Swiss-born, foreign-born and mixed clusters. As our study
177 sample included, by design, more HIV-infected and patients with drug-resistant TB
178 (9), we calculated weights to take sampling proportions into account. As a sensitivity
179 analysis, we restricted the analysis of clustering proportion to the patients in the
180 random sample from the TB registry (n=288) (9). All statistical analyses were
181 performed in Stata version 14 (Stata Corporation, College Station, TX) and R 3.1.2
182 (36).

183 In addition, we plotted the mean genetic distances (in SNPs) versus the mean
184 geographical distances (in km) of all patient pairs in a molecular cluster (distance
185 between the birth countries' capital cities). Plots were generated with the ggplot2
186 library in R (37).

187

188 **Ethics approval**

189 The study was approved by the Ethics Committee of the Canton of Bern,
190 Switzerland (9). Informed consent was obtained from all patients enrolled in the
191 SHCS. For patients outside the SHCS, informed consent was obtained by the
192 treating physicians. In some cases, informed consent could not be obtained from
193 the patient, because he or she could not be located or was known to have died.
194 For these cases, we obtained permission from the Federal Expert Commission on
195 Confidentiality in Medical Research to use the data provided by the treating
196 physician.

197

198

199 RESULTS

200

201 Study population

202 The study population consisted of 520 TB patients from the nationwide study in
203 Switzerland (9, 25, 27, 28, 38). The patient characteristics are described in [Table 1](#). A
204 total of 119 (22.9%) patients were born in Switzerland and 401 (77.1%) abroad.
205 Median age was 36.5 years (Interquartile Range 28–51). Overall, 113 (21.7%)
206 patients were HIV positive. Pulmonary TB accounted for 387 (74.4%) of all cases and
207 extrapulmonary TB for 133 (25.6%) ([Table 1](#)).

208

209 Transmission clusters

210 *Whole genome sequencing*

211 Isolates were sequenced with a median sequencing depth of 130x (range 22-
212 274x). For quality assurance, we compared laboratory-assay-based phylogenetic
213 lineage classification and spoligotyping pattern with the results generated from the
214 WGS data using KvarQ (9). We found 100% agreement between the two methods for
215 lineage identification and up to two discordant spacers in the spoligotyping patterns.

216 *Identification of molecular clusters based on WGS*

217 In the 35 previously defined MIRU-VNTR clusters, we found pairwise genetic
218 distances of 0 to 224 SNPs (median: 21.5 SNPs) ([Figure 1](#) and [Supplementary](#)
219 [Figure 1](#)). In the largest cluster (eight isolates), genetic distances were ≥ 54 SNPs.
220 Seventeen of 35 (48.6%) MIRU-VNTR clusters consisted of pairs separated by ≤ 12
221 SNPs, i.e. were confirmed as true transmission clusters, corresponding to 43 of 90
222 patients (47.8%) ([Table 2](#)). The remaining 18 clusters harbored isolate pairs
223 separated by >12 SNPs (47 patients).

224 The overall clustering proportion decreased from 17.3% (95% confidence
225 interval [95% CI]: 14.2-20.8%) based on standard genotyping (spoligotyping and
226 MIRU-VNTR), to 8.3% (95% CI: 6.0-11.0%) based on WGS. When restricting the
227 analysis to the 288 randomly selected patients, we found 27 clustered patients in 11
228 genome-based clusters, resulting in a clustering proportion of 9.4% (95% CI: 6.3-
229 13.3%). When using a more stringent WGS definition for transmission clusters (at
230 least one isolate pair in a cluster ≤ 5 SNPs distance), 13 of 35 (37.1%, CI: 21.5-
231 55.1%) MIRU-VNTR clusters were confirmed. These 13 transmission clusters
232 included 35/520 patients, corresponding to a clustering proportion of 6.7% (95% CI:
233 4.7-9.2%).

234 *Infection with multiple M. tuberculosis strains*

235 In five isolates that were part of transmission clusters defined by MIRU-VNTR,
236 we detected multiple alleles at several MIRU-VNTR loci, potentially indicating
237 infection with multiple *M. tuberculosis* strains. We therefore conducted an allele
238 frequency analysis based on sequencing reads for each SNP call. Despite the
239 presence of multi-allelic variant calls in all isolates (potential microevolutionary
240 events), none of the five isolates with multiple MIRU-VNTR bands showed evidence
241 of lineage- or sublineage-specific markers with multiple alleles in the sequencing
242 reads.

243

244 **Molecular clustering in Swiss-born, foreign-born and HIV-positive patients**

245 Four MIRU-VNTR clusters involved Swiss-born patients only, 16 clusters
246 foreign-born only and 15 clusters were of mixed birth group origin. Three of four
247 clusters (75.0%) involving only Swiss-born patients were confirmed by WGS as true
248 clusters (8/10 [80.0%] clustered Swiss-born patients). On the other hand, only 4/16

(25.0%) immigrant clusters (born on the same continent) were true clusters (8/37 [21.6%] patients). Of the 15 mixed clusters, 10 (66.7%) were true clusters (27/43 [62.8%] clustered patients) ([Table 2](#)). We assessed whether foreign-born patients were overrepresented in MIRU-VNTR clusters not confirmed by WGS. Among all 90 patients from the MIRU-VNTR clusters, foreign-born patients were more likely in clusters not confirmed by WGS compared to true clusters (aOR 4.5, CI: 1.5-13.6 $p=0.008$) ([Table 2](#)).

We then calculated the true (genome-based) clustering proportion for both Swiss- and foreign-born patients. The clustering proportion among Swiss-born cases decreased only slightly, from 19.3% (23/119, 95% CI: 12.7-27.6%) using MIRU-VNTR to 14.3% (17/119, 95% CI: 8.5-21.9) using WGS data. In contrast, the clustering proportion among immigrants was more than halved, from 16.7% (67/401, 95% CI: 13.2-20.7) to 6.5% (26/401, 95% CI: 4.3-9.4). [Figure 2](#) summarizes the possible factors leading to an overestimation of *M. tuberculosis* transmission based on standard genotyping, among foreign-born and native TB patients in low TB incidence settings.

The median genetic distance differed significantly between the three groups: 9 SNPs (range 8-15) in clusters with Swiss-born individuals only, 2 SNPs (range 0-162) in mixed clusters, and 24 SNPs (2-224) in clusters with foreign-born individuals only ($p=0.030$, [Figure 3](#)).

Geographical and genetic distances within molecular clusters

Plotting the mean genetic distance (in SNPs) versus the mean geographical distance between patient origins (capital cities) for each molecular cluster further supported the different patterns of clustering by birth origin of the patients ([Supplementary Figure 2](#)). Among foreign-born patients, a majority of MIRU-VNTR

275 clusters (11/16, 68.8%) consisted of patients born within 3,500 km of geographic
276 distance, but harboring genetic distances of >12 SNPs. This indicates that genetically
277 closely related strains, circulating in a geographically restricted area in the region of
278 origin, were imported to Switzerland. Among the mixed MIRU-VNTR clusters, for
279 which transmission is expected to have happened in Switzerland, a majority of
280 clusters (9/15, 60%) had mean geographic distances above 3,500 km, but genetic
281 distances of ≤ 12 SNPs, indicating recent transmission in Switzerland (Supplementary
282 Figure 2).

283

284 **Risk factors for transmission**

285 Female (aOR 0.39, CI: 0.18-0.85) and HIV-positive patients (aOR 0.39, CI: 0.16-
286 0.93) were significantly less likely to be involved in true transmission clusters (Table
287 1). In contrast, patients with cavitory disease were more likely to be associated with
288 transmission (aOR 2.31, CI: 1.05-5.10). There was a weak evidence for an
289 association between being born in Switzerland and being involved in a true
290 transmission cluster (aOR 2.21, 95% CI: 0.88-5.52 for Swiss-born compared to
291 foreign-born patients). Overall, the risk factors for transmission remained similar to
292 those previously reported when using standard genotyping methods (9).

293

294 **DISCUSSION**

295

296 In this nationwide study on 520 TB patients, 18 of 35 transmission clusters
297 identified by standard molecular genotyping (spoligotyping and MIRU-VNTR) were
298 refuted by WGS. This suggests that transmission of *M. tuberculosis* is generally
299 overestimated in low TB incidence countries such as Switzerland. Furthermore, we
300 found a striking difference between transmission clusters involving Swiss-born cases
301 and clusters involving foreign-born patients. WGS confirmed three quarters of the
302 clusters involving Swiss-born individuals only, but only one quarter of clusters
303 involving foreign-born patients only, hence indicating that transmission was especially
304 overestimated among the immigrant population.

305 *M. tuberculosis* strains from immigrants, which were defined as clustered by
306 MIRU-VNTR, but not by WGS, are likely genetically closely related genotypes,
307 imported independently from a high-incidence region, where they are highly prevalent
308 (19). Such strains accumulate genetic mutations over time (often leading to pairwise
309 SNP distances of >12 SNPs), but the MIRU-VNTR pattern may not change. In such a
310 situation, identical MIRU-VNTR will be wrongly interpreted as recent transmission in
311 the country of immigration (9). Similar observations were made in the UK, where
312 immigrant TB cases were identified in transmission clusters based on standard
313 MIRU-VNTR genotyping, although no epidemiological link could be found during
314 contact investigations (16).

315 The clustering proportion (indicating recent transmission) among Swiss-born
316 individuals was similar using standard genotyping and WGS, but more than two-fold
317 lower among foreign-born individuals when using WGS. In reality, the clustering
318 proportion among foreign-born individuals might even be lower, as we cannot
319 exclude that WGS-confirmed clusters (≤ 12 SNPs) involving immigrants might partly

320 represent transmission that happened in the country of origin and not in Switzerland.
321 Only social contact tracing could provide further insights into transmission dynamics,
322 but such investigations are notoriously difficult to perform, particularly among
323 immigrants (23, 39). The low proportion of true transmission clusters among
324 immigrants in our study was further supported by the weighted analysis of predictors
325 for transmission, which showed that foreign-born TB cases tended to be less likely
326 involved in true clusters compared to Swiss-born cases. Of note, the clustering
327 proportion among immigrants is remarkably similar to previous observations among
328 immigrant MDR cases diagnosed in Switzerland, which showed a clustering
329 proportion of 8% (compared to 7% in our study) based on standard genotyping and
330 contact tracing (30).

331 The majority of mixed molecular clusters as defined by MIRU-VNTR (i.e.
332 involving Swiss- and foreign-born individuals) showed small SNP distances (≤ 12
333 SNPs), confirming the intuitive explanation that transmission between Swiss-born
334 and foreign-born likely occurred in Switzerland. This was further supported by the
335 analysis of geographical distances between patient birth countries, which indicated
336 that most isolate pairs in the mixed clusters were from patients born far away from
337 each other, despite small genetic distances. The five mixed clusters harboring larger
338 SNP distances may reflect TB cases due to infections by circulating global or
339 European *M. tuberculosis* genotypes, such as the recently described large cluster in
340 Eastern Europe (40).

341 We found no evidence of infection with multiple strains among clustered TB
342 cases using the WGS data, despite the presence of double alleles in the MIRU-VNTR
343 patterns of five clustered isolates. Infections with multiple strains could potentially
344 also influence the identification of molecular clusters, as individual strains in an
345 infection with multiple strains cannot be resolved by MIRU-VNTR typing. The

346 prevalence and relevance of such multiple infections need to be further studied (41–
347 43).

348 A potential limitation of our study is the definition of transmission clusters by
349 WGS. The threshold of 12 SNPs, which we used to exclude transmission, has been
350 established by Walker *et al.* (17) and is in the range of what other studies have used
351 (21, 44, 45). However, an adequate cluster definition may be adapted according to
352 the setting (low versus high TB incidence), the study population and the technical
353 specifications of the WGS analysis pipeline (i.e. whether particular genomic regions
354 such as the PE/PGRS genes are excluded from the analysis, which was the case
355 here). For comparison, we repeated the analyses with a stricter definition of
356 transmission clusters also proposed by Walker *et al.* (MIRU-VNTR clusters in which
357 at least one pairwise distance was ≤ 5 SNPs (17)), which further reduced the number
358 of true clusters to 13, but did not change the overall clustering proportion significantly.
359 A further limitation may be our sample size: we included 12.3% of all TB cases
360 diagnosed between 2000 and 2008 in Switzerland, which potentially underestimates
361 the overall clustering proportion (29). Indeed, SNP distances could in fact become
362 shorter upon inclusion of additional patient isolates with intermediate genotypes,
363 hence increasing the proportion of true clusters.

364 In conclusion, only one quarter of foreign-born transmission clusters previously
365 identified by MIRU-VNTR were confirmed as true transmission clusters by WGS. We
366 therefore recommend the use of WGS for a more accurate identification of recent
367 transmission of *M. tuberculosis* among immigrants in low TB incidence countries, but
368 also in high TB incidence countries, where genetically closely related strains
369 circulate. Although analyzing WGS remains resource-intensive, the strategy adopted
370 in the UK documents that implementing WGS in the routine public laboratory
371 surveillance system is feasible (21, 46), and allows the prompt identification of

372 transmission clusters as well as information about the drug resistance genotype (46,
373 47). Our results also indicate that the native population in Switzerland may also play
374 a role in spreading TB, particularly individuals belonging to high risk populations (22,
375 23). Additional prospective studies using WGS are needed, possibly complemented
376 with social network analyses (20), to evaluate the usefulness of real-time analyses of
377 TB transmission dynamics in low TB incidence countries.

378

379

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435

436 **ACCESSION NUMBERS**

437 Raw sequencing data were submitted to the European Nucleotide Archive (ENA)
438 under project accession number PRJEB12179.

439

440 **CONFLICT OF INTEREST**

441 None to declare.

442

443

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- 639

640 **TABLES**

641 **Table 1.** Patient characteristics of tuberculosis (TB) cases diagnosed in Switzerland
 642 between 2000 and 2008, overall and comparing clustered and unclustered TB
 643 cases (unweighted), as well as risk factors for transmission (weighted analysis) as
 644 defined by genome-based molecular clustering.

Characteristic	Unweighted (n=520)						Weighted (n=4,221)	
	All cases n=520		Clustered n=43		Unclustered n=477		Adjusted Odds Ratio (95% CI) *	P-value
	n	(%)	n	(%)	n	(%)		
Age at TB diagnosis, years								0.46
16-29	154	(29.6)	14	(32.6)	140	(29.4)	1	
30-49	226	(43.5)	16	(37.2)	210	(44.0)	0.56 (0.22-1.41)	
≥50	140	(26.9)	13	(30.2)	127	(26.6)	0.71 (0.29-1.73)	
Sex								0.018
Male	254	(48.8)	26	(60.5)	228	(47.8)	1	
Female	266	(51.2)	17	(39.5)	249	(52.2)	0.4 (0.18-0.85)	
Country of birth								0.026
Switzerland	119	(22.9)	17	(39.5)	102	(21.4)	1	
Europe, without Switzerland	106	(20.4)	2	(4.7)	104	(21.8)	0.16 (0.03-0.83)	
Asia	120	(23.1)	3	(7.0)	117	(24.5)	0.24 (0.05-1.08)	
Sub-Saharan Africa	132	(25.4)	16	(37.2)	116	(24.3)	1.11 (0.36-3.45)	
Central and South America	20	(3.8)	3	(7.0)	17	(3.6)	1.99 (0.40-9.85)	
Other regions/Unknown	23	(4.4)	2	(4.7)	21	(4.4)	0.80 (0.13-5.02)	
Born in Switzerland								0.091
No	401	(77.1)	26	(60.5)	375	(78.6)	1	
Yes	119	(22.9)	17	(39.5)	102	(21.4)	2.21 (0.88-5.52)	
Residence status								0.41
Swiss nationals	130	(25.0)	14	(32.6)	116	(24.3)	1	
Foreigner/Residents	199	(38.3)	11	(25.6)	188	(39.4)	0.45 (0.17-1.23)	
Asylum seeker/Refugee	101	(19.4)	8	(18.6)	93	(19.5)	0.42 (0.11-1.56)	
Unknown	90	(17.3)	10	(23.3)	80	(16.8)	0.80 (0.22-2.81)	
HIV status								0.033
Negative	407	(78.3)	34	(79.1)	373	(78.2)	1	
Positive	113	(21.7)	9	(20.9)	104	(21.8)	0.39 (0.16-0.93)	
Site of disease								0.054
Extra-pulmonary TB	133	(25.6)	5	(11.6)	128	(26.8)	1	
Pulmonary TB	387	(74.4)	38	(88.4)	349	(73.2)	3.14 (0.98-10.07)	
Cavitary disease								0.039
No	407	(78.3)	28	(65.1)	379	(79.5)	1	
Yes	113	(21.7)	15	(34.9)	98	(20.5)	2.31 (1.05-5.10)	
Positive smear microscopy								0.049
No	352	(67.7)	21	(48.8)	331	(69.4)	1	
Yes	168	(32.3)	22	(51.2)	146	(30.6)	2.18 (1.00-4.73)	
TB in family or social surroundings in last 2 years								0.068
No	482	(92.7)	38	(88.4)	444	(93.1)	1	
Yes	38	(7.3)	5	(11.6)	33	(6.9)	2.95 (0.92-9.41)	

645 * Adjusted for age and sex, and weighted for sampling proportions

646 95% CI, 95% confidence interval; TB, tuberculosis

647 **Table 2. Number of MIRU-VNTR clusters and number of patients in MIRU-**
 648 **VNTR clusters confirmed by whole genome sequencing.** MIRU-VNTR clusters
 649 not confirmed by WGS ("false positive" clusters) and clusters confirmed by WGS
 650 ("true" clusters) are presented, according to the countries of birth of cases involved:
 651 clusters involving Swiss-born TB cases only, foreign-born cases only, or mixed
 652 clusters (e.g., involving both Swiss-born and foreign-born TB cases, or foreign-born
 653 cases from different continents).

	MIRU-VNTR clusters confirmed by WGS n (%)	MIRU-VNTR clusters not confirmed by WGS n (%)	Total number of clusters n
Molecular clusters *			
Swiss-born only	3 (75.0)	1 (25.0)	4
Mixed	10 (66.7)	5 (33.3)	15
Foreign-born only	4 (25.0)	12 (75.0)	16
Total	17 (48.6)	18 (51.4)	35
Patients in molecular clusters **			
Swiss-born only	8 (80.0)	2 (20.0)	10
Mixed	27 (62.8)	16 (37.2)	43
Foreign-born only	8 (21.6)	29 (78.4)	37
Total	43 (47.8)	47 (52.2)	90

654 MIRU-VNTR, mycobacterial interspersed repetitive-unit-variable-number tandem-repeat;
 655 WGS, whole genome sequencing

656
 657 * Fisher's exact test: p-value=0.031

658 ** Fisher's exact test: p-value <0.0001

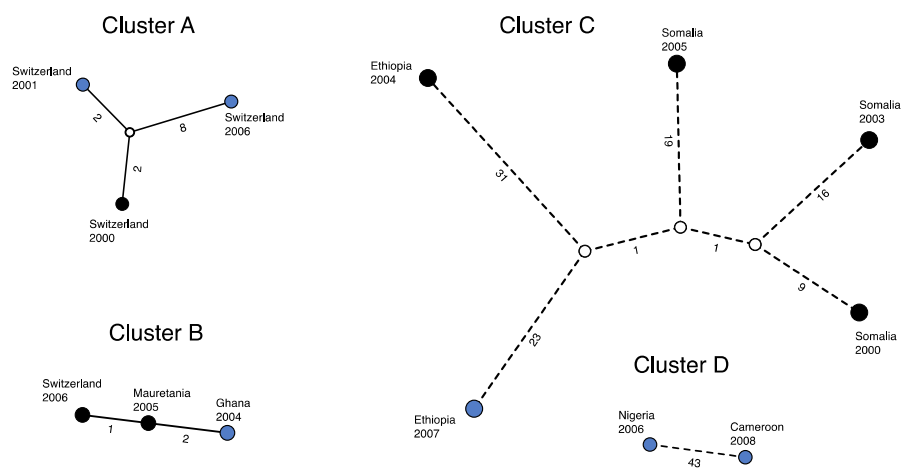
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661 **FIGURES**

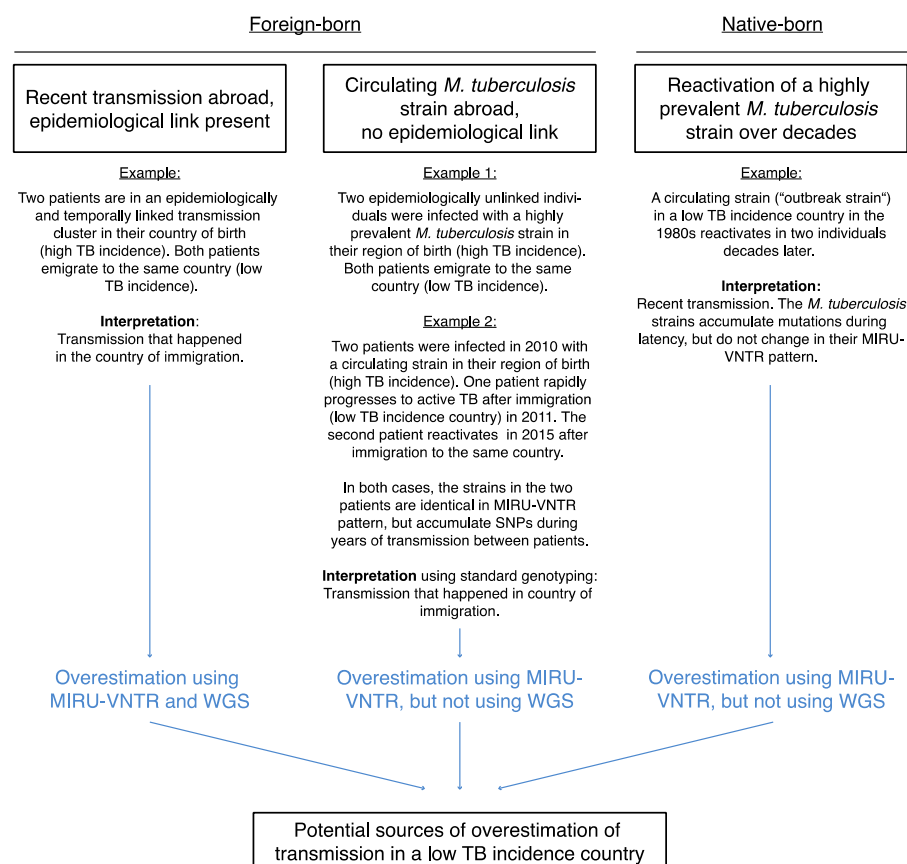
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663 **Figure 1. *M. tuberculosis* transmission network using SNP data from whole**
664 **genome sequencing.** Representative examples of different types of transmission
665 clusters that were previously identified by MIRU-VNTR: cluster A (all patients from
666 Switzerland) was confirmed as a true transmission cluster by WGS, with distances
667 of ≤ 12 SNPs between all isolates. Cluster B is a mixed cluster (one patient from
668 Switzerland and two patients from West Africa), which was confirmed to be a true
669 transmission cluster with one and two SNPs between patient isolates. Clusters C
670 and D were identified by MIRU-VNTR as transmission clusters, but WGS did not
671 confirm these clusters as true clusters (genetic distances of > 12 SNPs). Filled
672 circles represent patient isolates, white circles „median vectors“, i.e. hypothetical
673 isolates inferred from the sequencing data. Blue circles indicate HIV-positive
674 patients. Numbers next to lines indicate SNP distances. Countries of birth and years
675 of tuberculosis diagnosis are indicated next to circles. Clusters with solid lines are
676 “true” clusters, i.e. clusters confirmed by WGS (≤ 12 SNPs), whereas clusters with
677 dashed lines are clusters not confirmed by WGS (> 12 SNPs). All other transmission
678 clusters are shown in Supplementary Figure 1.



679
680

681 **Figure 2. Possible situations leading to an overestimation of *M. tuberculosis***
 682 **transmission in a low tuberculosis incidence country.**



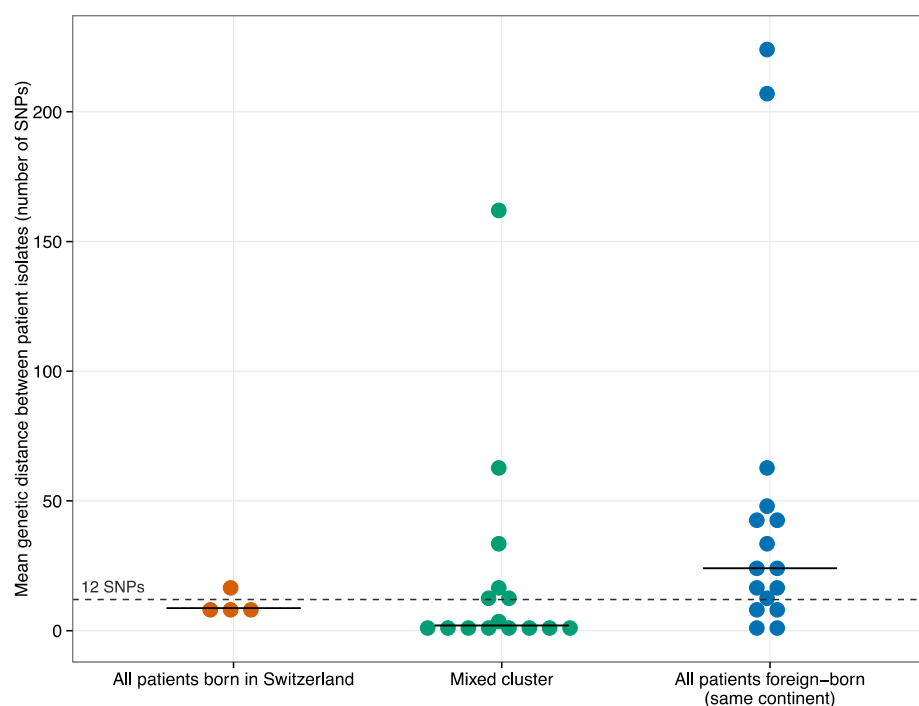
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684 MIRU-VNTR, mycobacterial interspersed repetitive-unit–variable-number tandem-

685 repeat; TB, tuberculosis; WGS, whole genome sequencing

686

687 **Figure 3. Median genetic distance in MIRU-VNTR-defined transmission**
688 **clusters.** Each data point shows the mean genetic distance (as number of single
689 nucleotide polymorphisms [SNPs]) in one of the 35 MIRU-VNTR-defined
690 transmission clusters. Solid black lines indicate median values of mean pairwise
691 distances within molecular clusters. The distribution of clusters with patients born in
692 Switzerland, mixed clusters and immigrant patients born on the same continent
693 (except Switzerland) was significantly different (Kruskal-Wallis non-parametric test,
694 $p=0.030$). The dashed line represents the cut-off for the definition of genome-based
695 molecular clusters (≥ 12 SNPs).



696

697